of the recombinant protein, the cells were suspended in 1% Tween 20-PBS, and then were disrupted by ultrasonication. After high-speed centrifugation, the supernatant was taken as the soluble fraction containing the recombinant protein. The soluble fraction was passed through a GSH-Sepharose 4B column (Pharmacia) and GST-pRB1, GST-pRB2, and GST-pRB3 the proteins, respectively, were absorbed on the column. The column was washed with WE buffer (10 mM 2-mercaptoethanol, 2 mM $MgCl_2$, 20 mM Tris-HCl pH 7.5), and then, the recombinant protein was eluted with G buffer (10 mM GSH, 50 mM Tris-HCl pH 9.6). Each eluted expression protein was dialyzed against a dialysis solution containing 50 mM Tris-HCl(pH 8.0) and 50% glycerin.

REMARKS

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 650-326-2400.

Respectfully submitted,

Joe Liebeschuetz
Reg. No. 37,505

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
Tel: (650) 326-2400
Fax: (650) 326-2422
JOL:pfh
PA 3199073 v1

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION

Paragraph beginning at line 34 on page 31 has been amended as follows.

The recombinant protein was produced as a fusion protein with GST when the pGEX vector is used. The recombinant protein was purified by taking advantage of the fact that this GST enzyme has an affinity for glutathione (GSH). After confirming the expression of the recombinant protein, the cells were suspended in 1% Tween 20-PBS, and then were disrupted by ultrasonication. After high-speed centrifugation, the supernatant was taken as the soluble fraction containing the recombinant protein. The soluble fraction was passed through a GSH-Sepharose 4B column (Pharmacia) and [GST-p53 (1-99) the protein was] GST-pRB1, GST-pRB2, and GST-pRB3 the proteins, respectively, were absorbed on the column. The column was washed with WE buffer (10 mM 2-mercaptoethanol, 2 mM $MgCl_2$, 20 mM Tris-HCl pH 7.5), and then, the recombinant protein was eluted with G buffer (10 mM GSH, 50 mM Tris-HCl pH 9.6). Each eluted expression protein was dialyzed against a dialysis solution containing 50 mM Tris-HCl(pH 8.0) and 50% glycerin.